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Remarks

The claims have not been amended in this Response, but a copy of the claims as currently pending is attached in an Appendix for the Examiner's convenience. Also, full citations for the various references discussed in this Response are provided in an Appendix. Copes of the full papers will be provided at the Examiner's request.

The claims are directed to methods for preventing and/or treating asthma by orally administering a composition consisting essentially of luteolin in an amount in a range of 0.1 to 10 mg/kg of body weight of the animal.

The characteristic features of asthma include airway hyperreactivity [Early Airway Response (EAR) and Late Airway Response (LAR)]; airway inflammation (Garlisi et al. 1997), and associated molecular parameters such increased levels of interleukin (IL)-4, IL-5 and IgE, and decreased levels of interferon-α (IFN-α) (Barnes, 2000). Ideally, these parameters are considered together to determine the asthmatic status of a patient.

Many of the rejections raised in the previous Office Actions are based on a belief that, since asthma is an inflammatory disease, it may be assumed that any anti-inflammatory compound would function as an anti-asthmatic drug, and, therefore, a teaching that luteolin has anti-inflammatory properties would be a teaching that luteolin was useful in treating asthma.

Applicants respectfully assert that this type of logic is flawed. Only certain anti-inflammatory compounds, such as steroids and anti-leukotrines, have been shown to have efficacy in treating asthma (Gupta et al, 2004). There are numerous anti-inflammatory drugs that are not used as anti-asthmatics, including aspirin, aspirin-like compounds, coxibs, and the like. Indeed, several of these drugs aggravate asthma (Simon, 2004), and are not advised for asthmatic patients.

Similarly, although histamine is believed to be involved in the development of asthma symptoms, it is not necessarily true that all histamine antagonists are useful in treating asthma. Several known histamine antagonists, clemastine, kitotifen and azelastine, have no significant effect on methacholine-induced bronchoconstriction in asthmatics (Nogrady and Bevan, 1978; Albazzaz and Patel, 1988, Cockcroft, 1992).

Increased levels of IFN-α are supposed to inhibit the allergic and asthmatic response. However, when an experiment was performed in which IFN- α levels were increased, a symptom

of asthma, airway hyperreactivity (AHR), increased in the mouse model. This was contrary to the expected results (Medoff et al., 2002).

IL-5 is believed to be an important biomolecule related to the development of AHR, but when an antibody against IL-5 was tested, it was not found to reduce asthmatic symptoms (Ichinose and Barnes 2004, Hendeles et al., 2004).

IL-4 is also one of the many cascades of the genesis of inflammation in allergy and asthma. However, it cannot be the sole responsible parameter for causing asthma, because it has been demonstrated (Hammelmann et al., (2000) that IL-4 deficient mice can also develop inflammation and asthma. Further, it is also observed that IGE, which is involved in the pathogenesis of asthma, is also produced independent of the IL-4 pathway (Finkelman et al., 1990).

Anti-asthmatic drugs developed so far do not inhibit just one mediator, but rather, are inhibiting several mediators and several pathways. For example, steroids inhibit release of many agents, such as histamine, leukotriene (LT)D4, LTC4, prostaglandin D2, and others.

It has been mentioned in the scientific literature that flavonoids have many beneficial effects, including anti-aging properties (Kim et al., 2004; Middleton et al. 2000), but many of these have potential toxic effects (Galati and O'Brien 2004). One of these, quercetin, is a flavonoid and anti-oxidant, and should have anti-aging properties. However, when experiments were done with quercetin in an animal model, it showed just the opposite effect, that is, it reduced the life span of mice (Jones and Hughes 1982). Although not directly related to asthma, this point is made to show that with complicated biochemical pathways, such as those involved in asthma, it is often difficult to make a leap from *in vitro* results on a particular biological marker to *in vivo* efficacy in an animal model.

Based on the above, Applicants respectfully assert that any novelty or obviousness rejection based solely on the fact that luteolin was discussed in the literature as being useful at raising or lowering the concentration of certain biomarkers, or in treating inflammation, but which failed to specifically disclose using luteolin in an animal model for asthma, should be withdrawn for failing to teach or suggest the invention as claimed. This would arguably be a tenuous extrapolation of what the reference actually teaches.

As discussed in more detail below, none of the cited references teaches or suggests the treatment of asthma in an animal model. It is clearly not the case that any compound that inhibits a particular mediator or mediators *in vitro* would be effective as an anti-allergic or anti-asthmatic drug. There are numerous inflammatory mediators (hundreds or more) implicated in the pathogenesis of asthma (Barnes et al., 1998). Histamine and hexosaminidase release are just two of these. It would not be obvious that an agent which inhibits only one or two of these would be likely to alleviate asthma. It is not valid to base an obviousness rejection on the fact that, *in vitro*, an agent has an effect on one or two of the mediators.

Applicants respectfully assert that there must be some experimental support for anti-asthmatic activity in the intact body, as well as an observation of side effects. Considering mere *in vitro* anti-mediator properties as the basis for concluding that the prior art taught that a compound is useful as an anti-asthmatic is not believed to be justified.

Rejections under 35 U.S.C. 102 (b)

Claims 1, 2, 4-8, 10, 12 and 13 have been rejected under 35 U.S.C. 102 (b) as anticipated by Aoyama et al. This rejection is respectfully traversed.

As discussed previously, Aoyama discloses an alcoholic extract of perilla seeds that include apigenin, chrysoeriol, luteolin and rosmarinic acid, and discloses that the extract is a histamine inhibitor.

The Examiner states that Aoyama teaches that the extracts can be isolated, and that fractions with the highest activity can be collected and used as histamine release inhibitors. However, Aoyama states that the fractions with the highest activity are not those with luteolin, but rather, apigenin.

Aoyama may teach that 0.5 to 30000 mg of an extract that includes apeginin may be useful for treating asthma, but fails to teach that 0.1 to 10 mg/kg of luteolin is effective for treating asthma.

Aoyama tested the alcoholic extract obtained from perilla seeds. This extract includes many compounds, including apeginin, chrysoeriol, and rosmarinic acid, in addition to luteolin. It is not clear how much activity is contributed by which individual component. Although Aoyama teaches that bioactive compounds contained in the extract can be concentrated, condensed, or

isolated, he does not teach what amount of luteolin has an anti-asthmatic effect, when orally administered at the dosage rate in the claimed method.

Aoyama further fails to teach whether any of the components possess biotoxicity, and therefore might be unsuitable as a drug. A medical pharmacological assessment of toxicity is imperative before concluding that a compound can be used as a drug, which Aoyama has not taught. In fact, apeginin, chrysoeriol, and rosmarinic acid have been found to possess mutagenic activities (Nagao et al., 1981). Also, Rosmarinus Oficianalis (rosemary), a primary source of rosmarinic acid, was found to be carcinogenic or toxic to mammals. (Johnson et al., 2001).

Aoyama's study was performed entirely *in vitro*, and the experiments were done in isolated human cultured mast cells rather than an intact body. An intact body, and not the *in vitro* or *ex vivo* isolated systems, is arguably required to test the efficacy and toxicity of a candidate drug in a complicated disorder such as asthma.

As an example, Smolinski and Pestka, while investigating the efficacy of three flavonoids as modulators of cytokine production, reported that inhibition of these two cytokines was observed in mice, but did not display the same patterns of inhibition as cell culture data. The results suggest that all three constituents possessed anti-inflammatory properties, but that cell culture data can only be used to approximate the potential effects in animals, and must be confirmed using appropriate animal models.

Further, Aoyama only observed the histamine inhibiting activity of the extract. However, the existing literature suggests that all histamine release inhibitors are not necessarily anti-asthmatic agents. For example, as reported by Bousquet et al (1992):

> "Histamine is an important mediator of asthma. The new anti-histamines, which block histamine at the H1-receptor level also possess some anti-allergic properties. At current dosages they appear to be safe. These drugs are effective in the treatment of rhinitis and conjunctivitis but their role in asthma is still under investigation. At a dose higher than usually recommended, they were shown to block exercise-induced asthma and, inconstantly, the early phase reaction after allergen challenge. Their effect on the late phase allergic reaction as well as their clinical efficacy during trials is, however, less consistent. The indication of H1-blockers in the treatment of asthma is therefore limited, especially since doses higher than recommended may lead to adverse reactions".

Further, it is unclear whether Aoyama intended for the luteolin to be administered orally, at the claimed dosage rate. If anything, Aoyama made a presumption, not supported by any valid experimental evidence or publication.

In the scientific discipline, making these kinds of presumptions and assumptions, such as on the basis of Aoyama's teachings, based on separate and unrelated *in vitro* experiments, is very common. However, these assumptions have to be evaluated by *in vivo* experimental conditions in the intact body, particularly with respect to complicated disorders such as asthma, which result from effects on a plurality of different biological pathways.

In vitro experiments of evaluating any compound show its effect on only one or a few parameters. In the body, thousands or more biological reactions are going on. A particular agent, that affects only one or a few parameters *in vitro*, may affect many other additional parameters in the body, which may or may not be favorable to alleviate the targeted disease. The agent will be active against the disease only when it really affects that particular targeted disease in the intact body. In many cases, it has been found that such generalized presumptions and assumptions were shown to be absolutely opposite to that of the experimental findings.

Accordingly, at best, Aoyama might have suggested performing additional research on luteolin, but did not disclose the claimed invention. Taken as a whole, luteolin was noted as one of the least active compounds, and as such, it would not have even been obvious to focus on luteolin over apigenin or the other compounds disclosed as being more active than luteolin.

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In light of the above points, Aoyama, which only disclosed *in vitro* tests, does not explicitly disclose the anti-asthmatic activity of luteolin in the intact body. Therefore, Aoyama's teachings merely extrapolate a theorized effect, but fail to provide a sufficient showing to anticipate the claimed invention.

Accordingly, Aoyama fails to teach the claimed invention, and Applicants respectfully request that the novelty rejection be withdrawn.

Claims 1, 2, 4-8, and 10-13 have been rejected under 35 U.S.C. 102 (b) as anticipated by Wang. This rejection is respectfully traversed.

First, Applicants respectfully assert that bronchitis is not the same as asthma (Blewase and Raymon, 2002). Wang experimented on patients with bronchitis, not patients with asthma. He did not define any clear anti-asthmatic activity of luteolin. For example, Wang did not disclose evaluating luteolin on a single characteristic feature of asthma, such as EAR, LAR, or even on the biochemical parameters such as histamine release, IL-4, IL-5, IFN- α and IGE in the serum or bronchoalveolar lavage fluid.

Therefore, the assertion that the functional effects for preventing the development of asthmatic features of LAR AND EAR, and of increasing IFN- α to a normal level and decreasing each of IL-4, IL-5, and IGE to a normal level, as well as inhibiting airway constriction and airway hyperreactivity are inherent to the method of treatment taught by Wang is an extrapolation at best. Applicants respectfully assert that Wang does not anticipate the claimed subject matter.

Claims 1, 2, 4-8 and 10-13 have been rejected under 35 U.S.C. 102 (b) as anticipated by Murai et al.

Murai tested certain lipoxygenase inhibitors such as luteolin and demonstrated some anti-allergic activity in the ear, but did not teach any method of treating asthma. As discussed above, not all anti-allergic agents are anti-asthmatic agents, and this could not have been confirmed without testing in an asthma model. It would not have been clear that luteolin had anti-asthmatic properties without *in vivo* testing.

The Examiner relies on the principle of inherency by stating that, since Murai administered the same drug at the same concentration, it inherently would have treated any

asthma that would have been present in any of the children. Applicants respectfully assert that the Examiner has misapplied the principles of inherency. That luteolin would treat asthma is not taught or suggested by Murai. If the Examiner's application of the principles of inherency were applied generally, there would be no claims available for new methods of using old drugs.

Accordingly, Murai fails to teach the claimed invention, and the rejection should be withdrawn.

Claims 1, 2, 4-8 and 10, 12, and 13 have been rejected under 35 U.S.C. 102 (b) as anticipated by Kotani et al. This rejection is respectfully traversed.

Kotani teaches that flavonoid compounds fisetin, quercetin, luteolin, and others are suitable for treating and preventing allergic diseases. Asthma is not specifically listed. Kotani did not test any compound in a living animal, only in *in vitro* experiments. As discussed above, the mere measurement of *in vitro* data is not sufficient in this instance to anticipate the claimed invention. Indeed, quercetin, one of the mentioned flavonoid compounds, has no anti-asthmatic effect in mice, but rather, made the animals very sick and led to their death. Therefore, although quercetin is a known PLA2 inhibitor and a strong anti-inflammatory flavonoid (Lee et al. 1982; Laumi and Becker 1985), it has an adverse effect and reduced the life span (Jones and Hughes, 1982).

In conclusion, Kotani fails to teach or suggest that luteolin is useful for treating asthma at all, let alone in the claimed mode of administration and dosage rate. Accordingly, Kotani does not anticipate the claimed invention.

Rejections under 35 U.S.C. 103 (a)

Claims 1, 2, 4-8 and 10-13 have been rejected under 35 U.S.C. 103 (a) as obvious over Murai in view of Tanaka and Nagai. This rejection is respectfully traversed.

Inventiveness of the Claimed Methods

Inventiveness lies in the new idea and the new method of carrying it out, when the idea has led to a new and advantageous result. Here, the new idea relates to the use of a plant-based, non-toxic compound in extremely low doses in an animal model of asthma, where luteolin

exhibited preventive and curative anti-asthmatic effects, before and after asthmatic manifestation in the animals.

While the method may appear very simple and obvious using impermissible hindsight, it is not so. The Applicants have carried out well-formulated laboratory experiments to elucidate the anti-asthmatic properties of luteolin. These experiments involved several steps, including sensitization with an antigen; development and measurement of allergen-induced airway constriction, EAR and LAR along the associated parameters of increased levels of IL-4, IL-5 and IgE, and decreased levels of interferon- α in the serum and bronchoalveolar lavage fluid (BALF). These characteristics were developed in a mouse model and luteolin was tested during the stages of development, as well as after the development of the asthmatic features, to evaluate its preventive and curative anti-asthmatic effect. The art made of record is silent regarding any such experiments.

There has been a long-felt need for finding a non-steroidal drug with negligible side effects for treating and/or preventing asthma. It is evident from the prior art that the problem had remained without a solution for a long time, and the claimed invention provides a solution to the problem.

Applicants have found that luteolin, a plant based compound, possesses anti- asthmatic activity (both preventive and curative) in intact, conscious, spontaneously-breathing mice, without exhibiting side effects. A good, working animal model, and not *in vitro* or *ex vivo* isolated systems, is required for testing the efficacy and toxicity of a candidate drug in an asthma model. If the activity of a candidate drug is tested on deceased animals or in isolated organs such as the trachea, it is not possible to observe and determine both the efficacy and toxicity of the compound, because the pharmacological effects of a drug is not confined to a single isolated system.

In the scientific literatures drawing this kind of extrapolation or assumption from far-fetched in-vitro experiments from cell culture and tissue from dead body is very common. Because *in vitro* experiments of evaluating any compound show its effect on only one or few tested parameters. In the body thousands and more biological reactions are going on. The agent that affects only a few parameters or biochemicals *in vitro* may affect many other additional parameters also in the body that may or may not be favourable to alleviate the targeted disease.

The agent will be anti-disease only when it really alleviates that particular targeted disease in the intact living body. Moreover, any inhibitor of inflammatory enzymes is not anti-asthmatic. For example, cyclooxygenases (COXs) are enzymes which cause severe inflammation in the body, but their inhibitors are not anti-asthmatic; and not only this, they aggravate asthma and instructed not to give bronchosensitive/ asthmatric patients (Namazy and Simon, 2002, Simon, 2004).

Analysis of the Cited References

Murai has been discussed above.

Nagai disclosed using a medicinal preparation, including several compounds, to treat dermatitis in the ears of mice, and investigated the effect of luteolin on histamine release from mast cells. It is not known how much activity was contributed by which constituent component. Further, while histamine release may be associated with many diseases, histamine release inhibitors used in any other system or *in vitro* are not necessary antiasthmatic. For example, histamine antagonists clemastine, ketotifen, and azelastine have no significant effect on methacholine-induced bronchoconstriction in asthmatics (Nogrady et al. 1978; Albazzaz and Patel; 1988 Cockcroft 1992).

Tanaka was cited for teaching that luteolin inhibits the activity of hexosaminidase release from mast cells, inhibits the release of histamine from mast cells or basophils, and suppresses cysteinyl leukotriene synthesis.

Applicants note that the Examiner has extrapolated the fact that luteolin was shown to have certain effects on certain biochemical pathways as rendering obvious the use of luteolin for all disorders that have been associated with a similar biochemical pathway. As discussed in detal below, this is not the case.

This logic seems to imply that all that needs to be shown to satisfy an obviousness rejection is that a drug has an affect on any relevant pathway that is ultimately associated with treating a particular disorder, even if treatment of the particular disorder was not taught. This is not, and should not be, the law, particularly with respect to disorders mediated by multiple biochemical pathways.

In conclusion, the combined teachings of these references do not render obvious the claimed invention.

Claims 1, 2, 4-8 and 10-13 have been rejected under 35 U.S.C. 103 (a) as obvious over Kotani in view of Tanaka and Nagai. This rejection is respectfully traversed.

As with the prior obviousness rejection, Kotani does not teach a method of treating asthma. The same comments presented above apply to this rejection as well.

Conclusion

On the basis of the arguments raised herein, Applicants respectfully assert that the invention as claimed is novel and non-obvious over the cited art. Withdrawal of the pending rejections, and allowance of the claims, is respectfully requested. The Examiner is encouraged to contact the undersigned if any questions remain.

Respectfully submitted,

Date: 8/18/06

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Appendix B - Cited References

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